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Hot water outperforms UV-C and commercial cleaning agents and disinfectants in reducing *Listeria monocytogenes* on woven conveyor belts

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ABSTRACT

Conveyor belts are recognized as common niches for persistent *Listeria monocytogenes* contamination in food processing environments. This study evaluated the efficacy of 24 commercially available cleaning and disinfection (C&D) agents, UV-C irradiation, and heat treatments against *L. monocytogenes,* using a laboratory biofilm model on woven conveyor belt material. Selected treatments were further tested on a used conveyor belt from food industry harboring a persistent outbreak strain.

Cleaning followed by disinfection, using concentrations recommended by manufacturers, achieved limited reductions (1.2–2.5 log) of *L. monocytogenes* after 10 min exposure in the laboratory biofilm model. Extreme concentrations of cleaning agents improved efficacy in the laboratory model but failed to eliminate *L. monocytogenes* from the used belt. All C&D agents applied at recommended concentrations led to >3.8 log reduction against a dried suspension of *L. monocytogenes* on stainless steel.

UV-C treatment resulted in dose-dependent but modest reductions (≤2.4 log) and the combination of C&D and UV-C improved efficacy in the laboratory model, but failed to eliminate *L. monocytogenes* from the used belt from food industry.

Heat treatment in a water bath at 60 °C for 1 h completely eliminated *L. monocytogenes* (>4 log reduction, negative after enrichment) from both types of conveyor belts, whereas hot dry air was less effective. Extended enrichment times revealed prolonged lag phases of stressed cells, highlighting the risk of false-negative results when standard detection protocols are applied.

Overall, the findings highlight the potential of hot water treatment as an effective strategy for eliminating *L. monocytogenes* from hard–to reach sites where chemical C&D and UV-C treatments are insufficient.

1. Introduction

Listeria monocytogenes is a food-borne pathogen associated with listeriosis, a disease with high mortality particular among vulnerable groups. Listeriosis is most commonly linked to ready-to-eat (RTE) foods such as cold smoked fish, soft cheeses, deli meats and fresh produce (Lopez-Valladares et al., 2018). In most cases, the food is contaminated during processing as a result of transfer of *L. monocytogenes* from unclean equipment/environment. It has been widely documented that *L. monocytogenes* can establish itself in niches within processing plants and persist for years (Belias et al., 2022; EFSA Panel on Biological Hazards (BIOHAZ) et al., 2024), and that persistent strains may also subsequently cause outbreaks (EFSA Panel on Biological Hazards (BIOHAZ) et al., 2024; Lachmann et al., 2022; Møretrø et al., 2026).

L. monocytogenes can be parts of biofilms which are very difficult to eradicate (Alvarez-Ordóñez et al., 2019). Typically niches where *L. monocytogenes* can persist include areas with limited accessibility, worn or damaged materials with crevices/scratches, or porous materials, often in combination with moisture (Tompkin, 2002) (EFSA Panel on Biological Hazards et al., 2024). Persistence of *L. monocytogenes* is

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commonly associated with drains and floors. Regarding food contact surfaces, persistence is most commonly found on conveyor belts and various types of machinery (Belias et al., 2022; EFSA Panel on Biological Hazards (BIOHAZ) et al., 2024).

The food industry depends on cleaning and disinfection (C&D) to produce safe food with high quality. A wide variety of cleaning agents and disinfectants are in use globally (Langsrud et al., 2026; Hamilton et al., 2025). The selection of cleaning agents is based on many factors such as the amount and type of soil, water hardness, surface material, health and environmental issues, regulatory requirements and costs. In a recent survey among 130 producers of RTE foods in 15 countries across five continents, alkaline cleaners (often with chlorine) were the most commonly used cleaning agents, followed by acidic cleaners, and peracetic acid-based agents and quaternary ammonium compounds (QAC) were the most widely used disinfectants (Langsrud et al., 2026). Despite routine application of C&D, *L. monocytogenes* is frequently detected on food contact surfaces, highlighting the limitations of conventional sanitation practices, particularly on difficult to clean sites.

Limited information is available in the scientific literature about the effects of cleaning agents in the food industry. Furthermore, standardized tests for validating their effects are lacking, in contrast to tests validating the effect of disinfectants (Anonymous, 2001, 2019). Regarding laboratory studies on the effect of C&D against *L. monocytogenes*, most studies focus on the disinfection step and not the cleaning step (Arthur et al., 2025; Hamilton et al., 2025). In prior studies, we showed that *L. monocytogenes* in biofilms in a woven conveyor belt were partly protected by the cleaning and biocidal effects of C&D (Fagerlund et al., 2020; Fagerlund et al., 2017).

A number of studies have assessed the effect of disinfectants on *L. monocytogenes* in laboratory studies (Arthur et al., 2025). The relevance of many of these laboratory studies to practical industrial settings is limited. Studies on stainless steel and polystyrene (microtiter plates) dominate, and although stainless steel is frequently used in the food industry, both stainless steel and polystyrene are non-porous materials that are typically easier to clean and disinfect than porous or rough surface materials (Ohman et al., 2024). Furthermore, many studies on the effect of disinfectants do not include a prior cleaning step (Arthur et al., 2025), which is always recommended in the industry, as disinfectants are intended to be used on cleaned surfaces. Another limitation is that often commercial agents are not used, but just the main active components. C&D-agents contain additional ingredients that will enhance their effect, e.g. detergents and chelators. Despite daily C&D, *L. monocytogenes* is still commonly isolated after C&D in the food industry (EFSA Panel on Biological Hazards (BIOHAZ) et al., 2024; Fagerlund et al., 2022).

Alternative or complementary mitigation strategies, including UV-C and heat treatments have received increased attention. UV-C (100-280 nm) is used in the food industry for the inactivation of microorganisms on foods as well as on food processing surfaces (Dhaliwal et al., 2025; Kim et al., 2016; Urban-Chmiel et al., 2025). The advantages of UV-C applications for processing surface treatments include ease of use at relatively low cost and the absence of odor or chemical residues after treatment. In addition, UV-C is generally permitted for hygienic applications in food-processing facilities; however, its use for direct treatment of foods is subject to regulatory restrictions depending on the application and jurisdiction. Known drawbacks include the inhibition of UV-C efficacy by food soils (Bintsis et al., 2000) and its limited ability to penetrate certain materials, for example inside machines, opaque food matrices, or worn or rough surface structures. Reported effects of UV-C on *L. monocytogenes* in existing biofilms also vary considerably, ranging from little or no effect to reductions >5 log (Hessling et al., 2025). However, nearly all studies have been conducted on stainless steel surfaces and only under laboratory conditions. Thus, data on the suitability of UV-C as part of effective *L. monocytogenes* mitigation strategies applied for contaminated processing surfaces under various relevant conditions are scarce.

Heat treatment of equipment or niches harboring persistent *L. monocytogenes* is recommended in some guidelines (FSIS, 2014; Tompkin et al., 1999). Although scientific evidence from industrial-scale experiments is limited, steam cookout of the floors in mushroom growing units has been shown to minimize the risk of contamination of subsequent batches with *L. monocytogenes* (Pennone et al., 2020), and heat treatment of meat chillers reduced the presence of *Listeria* sp. (Eglezos & Dykes, 2011). In practice, the use of heat on machines may be limited by the size of the machines and by low heat tolerance of certain machines/equipment components. In contrast, treatment of knives by immersing in hot water is common in the meat industry (Taormina & Dorsa, 2007).

In the present study, a range of commercially available C&D agents, covering the main C&D types used in the food industry, were tested against *L. monocytogenes* laboratory biofilms formed on a woven conveyor belt, as well as on a used woven conveyor belt from food industry harboring a persisting outbreak strain of *L. monocytogenes*. Also, the effects of heat and UV-C treatments were tested using the same experimental models.

2. Materials and methods

An overview of all experimental models, treatments, analytical approaches, and main findings is provided in Supplementary Table S1.

2.1. Model test systems for L. monocytogenes contaminated surfaces

2.1.1. Laboratory biofilm model of L. monocytogenes on new conveyor belt

Most control measures were evaluated using a laboratory biofilm model of *L. monocytogenes* on a new food-grade woven conveyor belt. The model used was modified from previous works (Fagerlund, et al., 2017, 2020). Briefly, overnight cultures (30 °C, 150 rpm, 16-18 h) in Brain Heart Infusion (BHI) broth (Oxoid, Basingstoke, UK) of seven *L. monocytogenes* strains (of various sequence types; ST7, ST8, ST9, ST18, ST19, ST121, ST394) were mixed in equal volumes and diluted to a final level of $\sim 10^6$ CFU/ml in BHI. The suspension was used as inoculum where 1 ml was added and partly submerging each coupon (1.5 × 1.0 cm) of a urethane-impregnated woven polyester fabric conveyor belt (E8/2 U0/V5 MT white FDA, Forbo-Siegling Transilon, Baar, Switzerland) placed vertically in a 24-well plate and incubated at 12 °C for 4 days, before being tested for mitigation measures as described below.

2.1.2. Used conveyor belt from food industry harboring a persistent L. monocytogenes strain

In addition to testing in the laboratory biofilm model described above, mitigation treatments were also tested on a used woven conveyor belt (4 years old) from a skinning machine at a cold-smoked salmon producer harboring a *L. monocytogenes* ST121 outbreak strain that had persisted for at least 12 months in the belt (Møretrø et al., 2026). In addition to general surface abrasion, the belt edges were locally frayed and showed minor cuts in several areas. The outbreak strain was detected on the belt after enrichment (Møretrø et al., 2026). The belt was then cleaned, disinfected, and stored in a plastic bag at 10 °C for four months. Coupons measuring 1.0 × 1.5 cm were cut from different sections of the belt with sterile scissors. 10 coupons were subjected to quantitative analysis and transferred to tubes with 4.5 ml Dey Engley neutralizing broth (Oxoid) with 2 g glass beads (2 mm, VWR). After vortexing, the tubes with the coupons were sonicated (Bransonic 3510, 40 kHz, 10 min), before plating to Rapid', L.mono (Labolytic, Trondheim, Norway). The level of *L. monocytogenes* was below the detection limit (45 CFU/coupon) for five of the coupons, while low numbers of *L. monocytogenes* (90-360 CFU/coupon) were detected on the remaining five coupons. When 10 other coupons were transferred to Half Fraser enrichment broth (Oxoid, 30 °C) and subsequently to Fraser broth (Oxoid, 37 °C), all 10 coupons tested positive for *L. monocytogenes*. Since the belt remained contaminated with *L. monocytogenes* and the bacteria had persisted on the belt for months, it was considered a relevant model for testing the effectiveness of control measures against a persistent *L. monocytogenes* strain. The level of *L. monocytogenes* was deemed too low to evaluate the reduction effect based on quantitative analysis, so it was decided to use a qualitative analysis instead. In this test, conveyor belt coupons after treatments were incubated in Half Fraser enrichment broth (30 °C) for at least three days. It was assumed that the detection limit was 1 CFU, meaning that a negative *L. monocytogenes* test indicated the bacteria had been completely eliminated from the conveyor belt coupon.

2.1.3. Laboratory biofilm model of L. monocytogenes on stainless steel

The *L. monocytogenes* inoculum was as described in section 2.1.1. Biofilms were formed by placing 2 × 2 cm stainless steel coupons (AISI304, 2B, Norsk stål, Oslo, Norway) into 50 mL Falcon tubes (Corning Inc., Corning, USA) to which 7 mL of the inoculum was added, followed by incubation at 12 °C for 4 days. The coupons with biofilms were used in the UV-C mitigation measures as described below (section 2.4).

2.2. Cleaning and disinfection

2.2.1. Cleaning and disinfection agents

Cleaning and disinfection agents to be tested were selected based on information from a previous study about the use of C&D agents among 130 food producers in a total of 15 countries (Langsrud et al., 2026). Products covering all major types of C&D agents used by these food producers were tested. Selection was also based on availability as most of the agents selected were obtained from suppliers in Norway. The C&D agents tested are shown in Table 1. As most producers reported using a single supplier of cleaning agents and disinfectants, most of the testing was done by pairing of a cleaning agent and a disinfectant from the same producer. For disinfectants with no cleaning agents obtained from the same supplier, the disinfectant was tested after cleaning with Enduro timesaver (alkaline) and after cleaning with Enduro Eco VE9 (acidic). The first phase of the testing was performed at the highest recommended user concentration (Table 1).

2.2.2. Cleaning and disinfection testing

2.2.2.1. Testing of C&D at recommended user concentrations. Coupons of conveyor belt material with a 4-day laboratory biofilm of *L. monocytogenes* were subjected to testing. All coupons were rinsed with 10 ml deionized water (dH_2O) before testing. Cleaning and disinfection agents were tested in sequence, with rinsing with dH_2O between treatments. Coupons of 1 × 1.5 cm were placed vertically in a 24-well microtiter plate. Cleaning agents with foaming ability were added using a foam pump bottle (Biltema, Oslo, Norway), allowing the foam to cover the coupon. For non-foaming cleaning agents and water (control), 2 ml liquid was added. After the cleaning step, the coupons were rinsed twice with 10 ml dH_2O. For testing of disinfection agents, the coupons were transferred and placed vertically in a new 24-well microtiter plate. Disinfectants with foaming ability were added using a foam pump bottle, allowing the foam to cover the coupon. For non-foaming agents and dH_2O (control), 2 ml liquid was added. Exposure to C&D agents was performed at room temperature. The C&D agents were tested at the highest recommended user concentration (Table 1), with an exposure time of 10 min. After the end of treatment, the coupons were transferred to tubes with 4.5 ml Dey Engley neutralizing broth with 2 g glass beads (2 mm). After vortexing, the tubes with the coupons were sonicated (40 kHz, 10 min), before making dilution series and spread plating to BHI and incubation at 30 °C. The experiment was performed in triplicate.

2.2.2.2. Testing at extreme concentrations of cleaning agents. In a prior study, it was shown that extreme concentration of cleaning agents could increase the effect of a C&D protocol against a biofilm of *L. monocytogenes* on woven conveyor belt (Fagerlund et al., 2020). Selected C&D agents were tested at extreme concentration in the present study. The test conditions were similar to the study with recommended user concentrations, except that cleaning agents were applied at concentrations about 10 times higher and with longer exposure times (30 min in each step). The experiment was performed in triplicate. The selected agents and conditions tested are listed in Table 2.

In addition to testing with the laboratory biofilm model, it was also assessed whether extreme concentrations of cleaning agents could eliminate *L. monocytogenes* from the used conveyor belt from food industry. After exposure and the sonication step in Dey Engley broth (as described above), the coupons were analyzed qualitatively for *L. monocytogenes* by transferring them to 10 ml Half-Fraser pre-enrichment broth, followed by Fraser broth (1-2 d incubation) and selective streak plating to Rapid' L.mono plates to assess the absence or presence of surviving *L. monocytogenes* (NMKL, 2007). The pre-enrichment was initially performed for 3 days at 30 °C. Samples not turning black on day 3 were incubated further and inspected (and transferred to Fraser broth

Table 1
Cleaning agents and disinfectants tested in this study.

Type/Product	Supplier	Type	Dilution (%)[a]
Cleaning agents			
Aqua CIP PH	Aquatic Chemistry AS	Acidic	2
Aqua Biocip	Aquatic Chemistry AS	Alkaline with chlorine	2
Aqua Foam Alkachlor	Aquatic Chemistry AS	Alkaline with chlorine	5
Enduro Timesaver	Lilleborg	Alkaline	5
Enduro Eco VE9	Diversey/ Lilleborg	Acidic	5
Addi Alkaskum 928	Lilleborg	Alkaline	10
Topaz MD3	Ecolab	Alkaline	5
Cleaning and disinfection			
P3 Topax 66	Ecolab	Alkaline with chlorine	3
Vixclor SE	Proquimia	Alkaline with chlorine	6
Deptal G	Kersia	Alkaline with chlorine	3
Disinfectants			
Hyposan	Arrow/Aquatic Chemistry AS	Hypochlorite	2.5
Aqua Des Foam PAA	Aquatic Chemistry AS	Peracetic acid	2
Diverfoam active VT70	Diversey	Peracetic acid	3
Suredis VT1	Diversey/ Lilleborg	Diamine[b]	2
Oxivir Plus	Diversey/ Lilleborg	H_2O_2	4
Enduro Plus VE6	Lilleborg	Hypochlorite	10
Topactive OKTO	Ecolab	Peracetic acid[c]	1
P3 Topax 91	Ecolab	QAC	3
P3 Topax 990	Ecolab	Diamine[b]	2
Asep 870	Proquimia	QAC	3
Perbac OPD	Kersia	Peracetic acid	1.3
Byotrol QFS	Byoworks	Polyhexamethylene biguanide hydrochloride (PHMB), Diamine[b]	5
Covipure	Last Viking Norway	Chlorine dioxide	Undiluted
e360 desinfeksjon	Boss Europe	Electrolysed water (hypochlorite)	Undiluted

[a] Highest recommended user concentration from manufacturer. Refers to dilutions of concentrates supplied from the manufacturers.
[b] Contains N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine.
[c] Also contains peroxyoctanoic acid.

Table 2
Tested combinations of cleaning (at extreme high concentration) and disinfection agents at extended exposure times (30 min in both steps) and their reduction effects on 4-day old *L. monocytogenes* biofilms on woven conveyor belt coupons.

No.	Cleaning step	Disinfection step	Number of replicates >4.0 log reduction[a]
1	40% Enduro Timesaver	3% Diverfoam active VT70	9
2	40% Aqua Foam Alkachlor	3% Diverfoam active VT70	8
3	20% Aqua Biocip +10% Aqua CIP PH	3% Diverfoam active VT70	5
4	20% Aqua Biocip	3% Diverfoam active VT70	6
5	40% Enduro Timesaver	7% Oxivir plus	9
6	40% Aqua Foam Alkachlor	7% Oxivir plus	8
7	40% Enduro Timesaver	20% Enduro Plus VE6	9
8	40% Enduro Timesaver	75% Ethanol	9
9	40% Aqua Foam Alkachlor	75% Ethanol	8
10	40% P3 Topax 66	1% Topactive OKTO	8

[a] A total number of nine replicates were performed for each combination.

if black) each day until day 6. The experiment was performed in duplicate.

2.2.2.3. European surface test of C&D agents on stainless steel. The susceptibility of *L. monocytogenes* cells dried on a stainless steel surface to C&D agents was tested in a modified version of the European surface test NS-EN 13697 (Anonymous, 2001). The tests were performed with a mixture of seven *L. monocytogenes* strains (as described above), obtained by mixing equal volumes of overnight cultures of the seven strains. Furthermore, the mixture was washed once with 0.85 % NaCl +0.1 % Tryptone, before mixing with a bovine serum albumin (BSA) solution (0.03 % BSA final concentration). After the addition of 50 µl of the bacteria/BSA to a 2 × 2 cm coupon of stainless steel (AISI 304), the cell suspension was allowed to dry in a safety hood at 37 °C before addition of 100 µl of the C&D agent to be tested. The C&D agents were tested individually only at the highest user concentration recommended by the manufacturers (Table 1). As controls, tests were performed with dH_2O instead of C&D agents. After exposure for 10 min, each coupon was transferred to a tube with 10 ml Dey Engley neutralizing broth, and sonicated (40 kHz, 10 min) to dislodge the cells, followed by spread plating to BHI. The experiment was performed in triplicate.

2.3. Heat treatments

Heat treatments were performed in a heated water bath and in a cabinet with heated air (for conveyor belt with persistent *L. monocytogenes* strain).

For treatments with hot water, coupons of conveyor belts (laboratory biofilm model and naturally contaminated belt) were put in glass bottles with 100 ml pre-tempered water that was incubated in a water bath (Thermo Scientific Precision GP28, Thermo Fisher Scientific, Waltham, USA). Survival of *L. monocytogenes* at different temperatures (50-70 °C) and exposure times were determined. The temperature of water inside the bottles was monitored.

For treatments with hot air, coupons of used conveyor belt from food industry were placed in a cabinet (Termaks, Bergen, Norway) at 80 °C for 24 h. Temperature and humidity during the heat treatment were measured with iButtons (iButtonlink, Maxim Integrated Products, San Jose, USA). Coupons of the conveyor belt stored at room temperature were included as positive controls.

After heat treatments, the coupons were analyzed for *L. monocytogenes* both quantitatively and qualitatively (after enrichment), as described above.

2.4. UV-C treatments

UV-C treatments were applied to conveyor belt and stainless steel coupons with *L. monocytogenes* (see section 2.1 for preparation) in a custom made aluminum chamber (1.0 × 0.5 × 0.6 m^3) equipped with two UV-C lamps (UV-C Kompaktleuchte, 2 × 95 W, BÄRO GmbH, Leichlingen, Germany) in the ceiling (Holck et al., 2018). The UV-C light, emitted essentially at 253.7 nm, was measured using a UVX Radiometer (Ultra-Violet Products Ltd., Cambridge, UK) equipped with a UV-C sensor (model UVX-25, Ultra-Violet Products Ltd., Cambridge, UK). Exposures were done at irradiation intensities of 5 mW/cm^2 or 10 mW/cm^2 by adjusting the distance between the UV-C lamps and the coupon surface to approximately 11 and 2 cm, respectively.

2.4.1. Coupons with laboratory biofilms

Prior to treatments, coupons were rinsed with 10 ml dH_2O to remove non-attached bacteria and excess liquid drained off by manual, careful shaking. The coupons were placed individually in petri dishes and treated at a power intensity of 10 mW/cm^2 for exposure times varying between 5 and 600 s (5-300 s for stainless steel), giving fluences between 0.05 J/cm^2 and 6 J/cm^2. One-side treated coupons were placed in a new Petri dish and treatment repeated for the other side of the coupon. An alternative power intensity of 5 mW/cm^2 but with the same treatment fluences was used in parallel treatments of selected samples. UV-C treated and non-treated (controls) coupons were transferred to glass tubes with 4.5 ml peptone water and 2 g glass spheres before whirl mixing for 1 min followed by sonication (40 kHz, 10 min). Suspensions were spread plated on BHI agar for *L. monocytogenes* quantification. To compare the antilisterial effects of UV-C treatments on intact biofilms versus biofilm cells being suspended prior to treatments, suspensions of the 4-day biofilm from conveyor coupons (see 2.1.1.) were also made by whirl mixing and sonication as described above. The suspensions were centrifuged, resuspended in peptone water before 50 µl (6-6.5 log CFU/coupon) were deposited on sterile, woven conveyor belt coupons and UV-treated as described above at a power intensity of 10 mW/cm^2.

2.4.2. Coupons of used conveyor belt from food industry

The used conveyor belt coupons naturally contaminated with *L. monocytogenes* (see section 2.1.2) were placed in petri dishes and UV-C treated at 6 J/cm^2 (power intensity of 10 mW/cm^2 for 600 s) on both sides as described above. After coupon treatments, enrichment for qualitative analyses of *L. monocytogenes* were performed: UV-C-treated and non-treated (controls) coupons were transferred to separate tubes with Half Fraser broth (30 °C). The tubes were incubated (maximum 8 days) until color change to black and then transferred to Full Fraser (37 °C) and further incubated (1-4 days). Full Fraser samples were plated on Rapid' L.mono (37 °C, 48h) for qualitative determination of *L. monocytogenes*.

2.5. Treatments combining cleaning & disinfection and UV-C treatments

2.5.1. Coupons with laboratory biofilms

The conveyor belt coupons were treated sequentially with C&D at their highest recommended user concentration followed by UV-C treatment. Coupons with biofilms were exposed first to a foam alkaline cleaner (Enduro Timesaver) for 10 min followed by rinsing with dH_2O then disinfection by a hypochlorite-based agent (Enduro Plus VE6) for 10 min at room temperature (see section 2.2.2). Rinsed coupons (twice in dH_2O followed by rinsing in Dey Engley neutralization solution) were placed in petri dishes and treated by UV-C (6.0 J/cm^2) on both sides as described above. Controls included coupons with biofilms treated with C&D only (10 + 10 min), water only (10 + 10 min) or UV-C only. *L. monocytogenes* levels on the coupons were quantified after sonication and plate count determination on BHI agar (37 °C, 48 h).

2.5.2. Coupons of a used conveyor belt from food industry

The coupons underwent the same treatment procedures (C&D, UV-C, or sequential C&D + UV-C treatment) as for the coupons with the laboratory biofilms. After treatment, the coupons were transferred to tubes with Half Fraser broth (30 °C) followed by Full Fraser broth (37 °C) and streak plating on Rapid' L.mono agar for qualitative detection of *L. monocytogenes.*

2.6. Statistical analysis

All data were log-transformed before statistical tests. Statistical significance of differences in effect of treatments was tested using one-way ANOVA followed by Tukey's post hoc test for pairwise comparisons in Minitab v22 (Minitab Inc., State College, PA, USA).

For treatments with extreme concentrations of cleaning agents, we assessed whether the proportion of replicates achieving >4.0 log reduction differed between treatments. A 4 log reduction is stated as a requirement for disinfectants to be effective in surface tests (Anonymous, 2001). The comparison was performed using Fisher's exact test on 2×2 contingency tables (https://langsrud.com/fisher.htm), based on the number of replicates (out of 9) exceeding the threshold.

For the UV-C data, a general linear model (ANOVA, Minitab) was applied with log reduction as the response variable. Material (conveyor vs steel) and intensity (5 vs 10 mW/cm^2) were included as fixed factors, and fluence was treated as a continuous covariate to account for dose-response effects. Model assumptions were assessed using residual plots and normal probability plots. Significance was determined at $\alpha = 0.05$.

3. Results

3.1. Effect of cleaning and disinfection

3.1.1. Effect of cleaning and disinfection at recommended user concentrations

A total of 10 cleaning agents (including 3 products claiming a combined cleaning and disinfection effect) and 14 disinfectants were tested at their highest recommended user concentration (Table 1) for 10 min exposure time. Cleaning agents were tested both as a single-step treatment and in combination with a subsequent disinfection step. The average level of *L. monocytogenes* before treatments was 6.6 ± 0.4 log cfu per coupon.

A total of 42 different treatments (unique combinations of cleaning agents and disinfectants) were tested against the 4-day laboratory biofilm on conveyor belt. None of the cleaning or disinfectant agents showed statistically significant difference in reduction of *L. monocytogenes* in pairwise comparisons between all agents. The mean reduction of individual cleaning agents when tested without disinfectants was in the range 0.75-1.54 log (Fig. 1). The total effect of cleaning and disinfection was in the range 1.20-2.57 log reduction, while the effect of the disinfection step alone was in the range of 0.07-1.37 log reduction. For 2 of 25 combinations of cleaning agents and disinfectants, there were a statistically significant ($p < 0.05$) increased reduction by adding a disinfection step, both were hypochlorite disinfectants used after an alkaline cleaning step.

When comparing types of cleaning agents and disinfectants (see classifications in Table 1), it was increased effects ($p < 0.05$) of adding a disinfection step with QAC and peracetic acid based disinfectants, compared to a cleaning step only. Also, amine disinfectants had lower ($p < 0.05$) effects than QAC and peracetic acid-based disinfectants, when the effect of the disinfectant step alone was compared.

3.1.2. Effect of extreme concentrations of cleaning agents

A total of 10 combinations of C&D agents were tested at extreme concentrations with 30 min exposure time. When conveyor belt samples

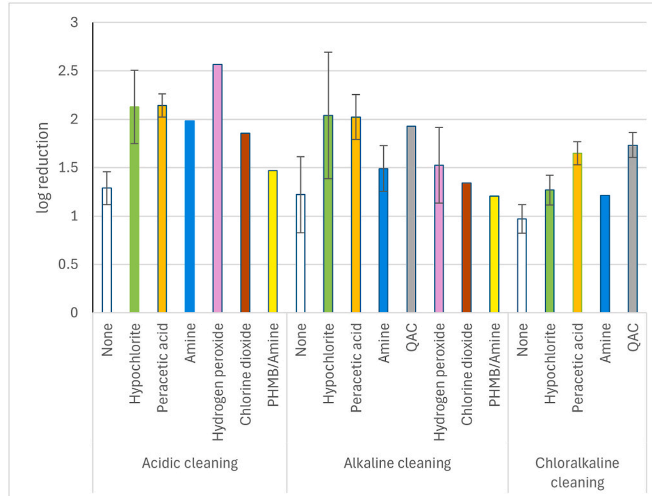

Fig. 1. Effect of cleaning and disinfection against *L. monocytogenes* in laboratory biofilms on a woven conveyor belt. A total of 42 different combinations of commercial cleaning agents and disinfectants were tested at their highest recommended user-concentration for 10 min. Log reductions means and standard deviations (where n > 1) are shown for groups of cleaning agents and disinfectants. "None", indicates that no disinfection was performed, thus only results from exposure to cleaning agents are shown.

with a 4-day laboratory grown biofilm were exposed to extreme concentration of cleaning agents followed by disinfection, >4 log reduction was obtained for eight of the treatments for at least 8 of 9 tested replicate coupons (Table 2). For the two treatments (no. 3 and 4) involving a cleaning step with 20% of an alkaline chlorine product, >4 log reduction was observed for 5 and 6 out of 9 tested coupons (Table 2). There was no significant difference in effect between treatments ($p > 0.05$) when pairwise comparisons were performed with Fisher's exact test.

For the used belt from food industry, even when applying extremely high concentrations of cleaning agents and relatively long exposure times (30 min for both cleaning and disinfection), *L. monocytogenes* was detected after enrichment in all three replicates for every treatment. Although the pathogen was eventually detected, the time to color change (black; indicating presence of *Listeria*) during pre-enrichment in Half-Fraser broth was longer than expected compared to the standard ISO 11290-1 procedure. Across 54 replicates of cleaning and disinfection treatments, the number of days (d) required for black color development after pre-enrichment were as follows: d3 = 7 treatments, d4 = 36, d5 = 6 and d6 = 1. In contrast, all 10 control samples treated only with water were black after 3 days.

3.1.3. European surface test of C&D agents

To evaluate the efficacy of C&D agents on *L. monocytogenes* cells dried on stainless steel, each agent was tested using the European surface test.

Individual exposure to all 24 C&D agents at their highest recommended concentration (see Table 1) for 10 min, resulted in no detectable surviving bacteria (>3.8 log reduction, except for one (2.8 log reduction) of three replicates with Byotrol.

3.2. Reduction of L. monocytogenes by surface UV-C treatments

UV-C treatments using 10 mW/cm^2 irradiation intensity showed higher ($p < 0.001$) effect against *L. monocytogenes* in laboratory biofilms on stainless steel (2.2-≥3.7 log reductions) compared to the woven conveyor belt (0.2-2.4 log reductions; Fig. 2). There were significantly increased log reductions ($p < 0.001$) with increasing dosage (fluence). For the conveyor belt, only the highest UV-dose tested (6.0 J/cm^2) gave significantly increased reductions in *L. monocytogenes* counts (2.4 log; p

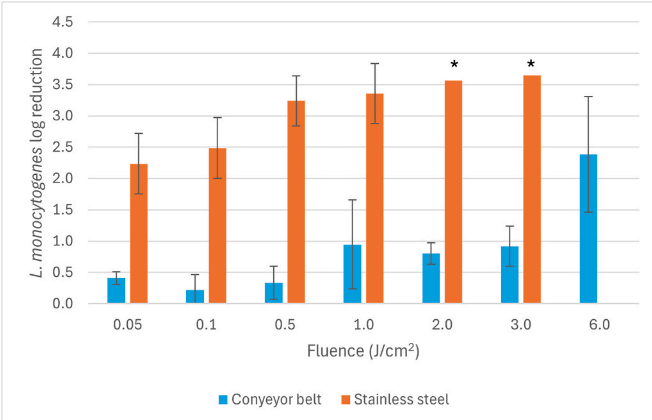

Fig. 2. Reduction of *L. monocytogenes* by different doses of UV-C-treatment (irradiation intensity 10 mW/cm^2) of laboratory biofilms on woven conveyor belt and stainless steel coupons. Means and standard deviations of 4 replicate experiments except for conveyor belt and stainless steel at 0.05 mJ/cm^2, where 2 and 3 replicate experiments were performed, respectively. UV-C dosages that led to reductions below the detection limit on stainless steel are marked with an asterisk. For these results the log reductions corresponding to the detection limit are shown, without standard deviation.

$<$ 0.05), compared to the other dosages where 0.2-0.9 log reductions were obtained. For stainless steel, reductions below the detection limit were obtained already at lower fluences and the 6.0 J/cm^2 UV-dose was not included (Fig. 2). There was no significant effect of UV-C intensity ($p = 0.51$) as similar reductions were obtained using half the UV-C intensity (5 mW/cm^2) with the same UV-doses applied through longer exposure times (Supplementary Fig. S1).

When UV-C treatments doses between 0.1 and 6.0 J/cm^2 were applied on conveyor belt coupons with added suspensions of *L. monocytogenes* biofilm cells (obtained by sonication; see section 2.4), no viable cells were detected in treated samples (data not shown). This represents reductions of ≥4 log.

3.3. Reduction of L. monocytogenes on conveyor belts by combining cleaning and disinfection and UV-C-treatments

Cleaning by an alkaline agent and disinfection by a hypochlorite-based agent at their highest recommended user concentrations followed by high dose UV-C (6.0 J/cm^2) treatment, gave 4.3 log reductions

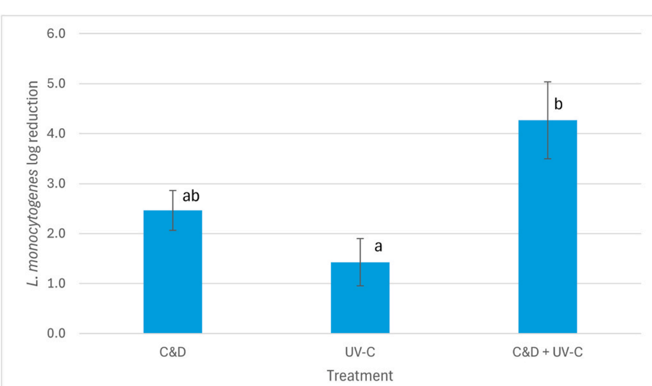

Fig. 3. Reduction of *L. monocytogenes* counts by cleaning (alkaline) & disinfection (hypochlorite) and UV-C-treatment (10 mW/cm^2; Fluence 6.0 J/cm^2) performed separately and sequentially (C&D followed by UV-C) on laboratory biofilms on conveyor belt material. Means of three replicate experiments (each with 3 parallels per treatment) and standard deviations are shown. Different letters (ab) above the bars indicate significant differences ($p < 0.05$).

of *L. monocytogenes* on the laboratory biofilm conveyor belt samples (Fig. 3). The effect of C&D followed by UV-C treatment was significantly higher ($p < 0.05$) than UV-C alone, but not significantly higher than C&D alone.

The same treatments of C&D and UV-C applied separately and as sequential treatments were tested on coupons of the used conveyor belt from food industry. The *L. monocytogenes* level in this belt was too low to determine quantitative effects of treatments, but following the treatments, *L. monocytogenes* was detected after standard enrichment in all parallels of both replicates for all treatments. For the biofilm coupons treated with both C&D and UV-C, the time until indication for growth of *Listeria* sp. (black colour) during enrichment in Half Fraser broth took several days. However, after 6-8 days incubation, the Half Fraser enrichment cultures turned black and the presence of *L. monocytogenes* in the treated samples was confirmed by plating on selective *Listeria* growth media (Rapid' L.mono).

3.4. Reduction of L. monocytogenes by heat treatment

For the laboratory conveyor biofilm model, exposure of coupons with a 4-day biofilm of *L. monocytogenes* in water bath at 60 °C for 1 h, lead to elimination of *L. monocytogenes* in all coupons tested (negative after enrichment), corresponding to >4.0 log reduction in the quantitative analysis (Table 3). All coupons exposed at 50 °C (1 or 2 h) were positive for *L. monocytogenes* in qualitative tests after enrichment. In the quantitative analysis, >4.0 log reductions were observed for coupons exposed to 50 °C for 2 h, while for coupons exposed at 50 °C for 1 h, the average reduction was 3.1 log.

For the used conveyor belt from food industry, submerging coupons with *L. monocytogenes* in a water bath at 60 °C for 1 h, eliminated (negative after enrichment) *L. monocytogenes* in all 10 coupons tested. The pre-enrichments from these samples were incubated >14 days, without signs of black precipitations. When coupons were exposed to water at 50 °C for 1 or 2 h, *L. monocytogenes* survived in all 10 + 10 coupons tested.

Treatments of the used conveyor belt with dry, hot air (<20% RH, 80 °C) for 24-28 h showed survival of *L. monocytogenes* (qualitative assessment after enrichment) in 9 out of 10 coupons.

It was observed that pre-enrichment broths from some of the coupons heat treated in the water bath at 50 °C or with hot dry air at 80 °C did not change color (to black) after 3 days pre-enrichment, however they turned black (indicating growth of *Listeria* sp.) after extended pre-enrichment times (up to 6 days) and were subsequently confirmed to be positive for *L. monocytogenes* by plating to selective medium. During the heat treatments in hot water or air, a total of 65 control coupons were kept at room temperature. Pre-enrichment broth of all these

Table 3
Effect on *L. monocytogenes* after exposure of conveyor belt coupons to heat in a water bath or in a cabinet with dry air.

	Water bath			Dry air[a]
	60 °C 1h	50 °C 1h	50 °C 2h	80 °C 24-28 h
Laboratory biofilm (quantitative, n[b] = 6)	>4.0[c]	3.1 (0.5)[d]	>4.0	NT[e]
Laboratory biofilm (qualitative, n = 6)	Survival 0/6[f]	Survival 6/6	Survival 6/6	NT
Used conveyor belt (qualitative, n = 10)	Survival 0/10	Survival 10/10	Survival 10/10	Survival 9/10

[a] Relative humidity >20%.
[b] n = number of coupons tested.
[c] Log reduction based on quantitative detection.
[d] Mean log reduction (standard deviation).
[e] NT; not tested.
[f] Number of coupons with survival/total number of coupons, based on qualitative detection of *L. monocytogenes* after enrichment.

coupons turned black within three days, and all samples were subsequently confirmed to be positive for *L. monocytogenes*.

4. Discussion

This study underscores the difficulty of controlling *L. monocytogenes* on woven conveyor belts using chemical cleaning and disinfection (C&D), consistent with previous findings (Fagerlund, et al., 2017, 2020). Conveyor belts are among the most common sites for *L. monocytogenes* persistence in food processing environments (EFSA Panel on Biological Hazards (BIOHAZ) et al., 2024). Although the tested C&D agents had limited effect at manufacturer-specified user concentrations on the conveyor belt, all tested C&D agents demonstrated high bactericidal activity at their recommended user concentrations when applied to dried bacteria on stainless steel, a non-porous surface. Thus, the failure appears not to be the efficacy of the agents themselves, but rather their inability to reach bacteria embedded within the belt structure. Porous materials present a barrier to effective C&D, likely due to limited penetration and reduced mechanical impact. Protection by biofilms may also have contributed to the limited effect of C&D. This underscores the importance of hygienic design in food processing equipment, particularly the use of non-porous materials that facilitate effective cleaning and reduce the risk of bacterial persistence in food processing facilities (EFSA Panel on Biological Hazards (BIOHAZ) et al., 2024; EHEDG, 2016).

Mechanical force, such as scrubbing or high-pressure rinsing, is generally considered essential for effective cleaning. However, no mechanical action was applied during the C&D procedures in this study. The contribution of mechanical force to remove bacteria embedded in porous materials remains uncertain. Notably, the used conveyor belt employed here, previously linked to persistence of an outbreak strain, continued to harbor *L. monocytogenes* despite extensive cleaning by the food producer, including scrubbing and pressurized water rinsing (Møretrø et al., 2026). This underscores the limitations of C&D when contaminants reside in inaccessible areas, even when mechanical force is employed.

The laboratory biofilm setup did not include food soils (e.g., fats, proteins), which are common in industrial environments and can influence cleaning and disinfection efficacy (Kragh et al., 2024). Food soils may shield bacteria from chemical agents, or inactivate active compounds, but can also facilitate removal during cleaning. The absence of soils in our tests may have resulted in conditions (e.g. limited neutralization of active compounds, more extreme pH conditions) potentially enhancing bactericidal effects compared to industrial settings. Also, our laboratory biofilm model utilized high concentrations of *L. monocytogenes* in a mono-species setup, which may not fully reflect industrial conditions where the pathogen typically occurs at low levels within mixed microbial communities (Møretrø & Langsrud, 2017). These limitations should be considered when interpreting the results. Future studies should incorporate food soils, mechanical action, and mixed-species biofilms to better simulate industrial C&D scenarios.

Although hundreds of C&D agents are marketed to the food industry, only a sub-set was tested in this study. While it cannot be excluded that other agents might perform differently, the products evaluated represent commonly used formulations from major international suppliers, supporting the relevance of our findings to industry practices. It should also be mentioned that we are not aware of other studies where such high numbers of commercial C&D agents have been tested against *L. monocytogenes*.

Extreme concentrations of cleaning agents markedly improved efficacy compared to recommended user concentrations, consistent with previous research on woven conveyor belts (Fagerlund, et al., 2017, 2020). These levels are not intended for routine use, as label instructions may define legal application and higher concentrations may pose health, equipment, and environmental risks. Results should therefore be viewed as experimental, not as industrial recommendations.

Although being regarded as critical for hygienic control in food industry premises, cleaning and disinfection have their limitations. Additional strategies therefore seem needed for efficient control and reduction or elimination of *L. monocytogenes* on risk surfaces/equipment in such environments. Heat treatment represents a promising alternative for controlling *L. monocytogenes*, particularly on porous materials. Although heat is widely applied for microbial control in foods, less is reported about its use for equipment sanitation, except in systems designed for high temperatures (e.g. CIP systems in dairies). Hot water, steam and dry heat have shown potential for surface decontamination (Crandall et al., 2010; Hua et al., 2021; Z. Hua & M. J. Zhu, 2024; Pennone et al., 2020; Tobin et al., 2020; Tompkin et al., 1999), but material properties, including thickness and heat tolerance, must be considered. A widely used guideline for control of *L. monocytogenes*, recommends steam treatment at a minimum temperature of 71.1 °C sustained throughout the equipment, with a holding time of 1 h or more. For heat-sensitive equipment, a lower temperature of 62.8 °C may be used, provided the exposure time is extended accordingly (Tompkin et al., 1999). The present study explored the use of a relatively mild hot water treatment, 60 °C for 1 h, which offers potential advantages in terms of energy efficiency and material compatibility compared to higher temperatures. However, despite its microbiological efficacy, the practical application of moist heat on conveyor belts requires caution. Repeated exposure of polymer based belt materials to elevated temperatures may accelerate material degradation, including microcracking and warping of hinges or pins, potentially reducing belt lifetime. In addition, implementation at industrial scale may be constrained by equipment design, production downtime, and energy use. Accordingly, implementation of heat treatment should be based on a balanced consideration of microbiological efficacy, hygiene outcomes, and material and operational constraints.

In this study, hot-water bath treatment (60 °C) proved more effective than dry heat (80 °C) for reducing *L. monocytogenes* on woven conveyor belt materials. In our previous investigation of a skinning machine involved in a *L. monocytogenes* outbreak, our initial suspicion that the woven conveyor belts served as harborage sites arose because, unlike the rest of the skinning machine, they had not been subjected to 80 °C dry heat treatment (Møretrø et al., 2026). In view of the results from the present study, it is likely that such dry heat treatment would not have eliminated *L. monocytogenes* from the belts anyway. The difference in bactericidal effects between hot water and dry air treatment likely reflects the superior heat transfer properties of water compared to air (Gu et al., 2023). The higher thermal conductivity and heat capacity of water enable rapid and uniform heat penetration into surface microstructures and microbial niches. In contrast, dry heat relies on air, which transfers thermal energy less efficiently. Additionally, moisture in hot-water treatments may enhance lethality by promoting protein denaturation and membrane disruption in bacterial cells. Further research should explore how surface topography, material composition, and heat transfer dynamics interact to optimize thermal sanitation strategies for food processing environments.

The anti-listeria effects of UV-C treatments of biofilms on the conveyor belt and stainless steel were significantly higher on the latter. The difference in effects is probably due to variation in surface structure between the two materials. In the more rough, structured and porous conveyor belt material, shadow effects are likely to occur, and *L. monocytogenes* are partly protected from being exposed to UV-C treatments compared to the more smooth stainless steel surfaces where such effects and protection is limited. The effects of UV-C reported under laboratory model conditions by other studies vary considerably from minimal inactivation (Kim et al., 2016), to a few log reductions (Bae & Lee, 2012), to complete elimination (Tajik et al., 2015). This probably reflects both differences in experimental set-up/procedures between studies and the various parameters influencing bactericidal action of UV-C irradiation (Z. Hua & M.-J. Zhu, 2024). Our study showed that originally biofilm-embedded bacteria retain their UV-C sensitivity as suspended cells. This implies that UV-C, although having limited effects on *L. monocytogenes* reductions in biofilms, provide effective listericidal effects once the bacteria are in a suspended/planktonic state and are effectively exposed to the UV-C light. This and similar knowledge on the limitations and possibilities for using UV-C in hygienic process control should be practiced to determine where cost effective UV-C treatment strategies could be implemented in the food production sector.

The hypothesis behind testing the effect of C&D followed by UV-C treatment is that C&D may make the biofilm-embedded bacteria more exposed and susceptible to the UV-C treatments. Although this sequential treatment provided higher *L. monocytogenes* reductions than UV-C alone, it did not produce reductions that were significantly higher than C&D alone. The tests were only performed using one selected combination of C&D agents (an alkaline cleaner and a hypochlorite-based disinfectant) and we cannot rule out whether other C&D agents could have achieved additive effects to UV-C treatments.

None of the treatments of the used conveyor belt from food industry using C&D and/or UV-C applied as single and sequential treatments were able to eliminate *L. monocytogenes* from the belt material. The *L. monocytogenes* in this belt seem to be more tolerant to such treatments than in the conveyor belt used in the laboratory biofilm model, even if the number of *L. monocytogenes* before the treatment was much higher in the latter. One possible explanation was that compared to the new conveyor material used in the laboratory model, the used belt was worn and this may have contributed to increasing the porosity of the material which led to uptake of *L. monocytogenes* deeper into the belt and thus increasing its protection against mitigation measures. Another possible explanation of increased protection of *L. monocytogenes* in this belt, could be the presence of additional bacterial species, compared to the mono-species laboratory model. It was previously shown that the belt contained only a minority of *L. monocytogenes* and was dominated by *Pseudomonas* spp. and *Enterococcus* spp. (Møretrø et al., 2026). Although this analysis was performed after the belt had been subjected to enrichment in Half-Fraser broth, it is likely that *L. monocytogenes* was also a minority of the total bacterial population before enrichment, as the enrichment procedure is designed to promote the growth of *Listeria* spp. and inhibit the growth of other bacteria. Strain-specific differences in stress tolerance are unlikely to account for the higher survival observed on the used belt. Although the outbreak strain carried the *qacH* gene, this determinant was also present in two of the strains in the laboratory cocktail, and truncated *inlA* variants were detected in both strain sets. This supports the interpretation that enhanced survival on the used belt primarily reflects factors such as material wear and biofilm-associated protection within a mixed microbial community rather than increased intrinsic tolerance of the outbreak strain.

In this study, a longer pre-enrichment period than specified in standard tests (ISO, 2017; NMKL, 2007) was required to achieve the characteristic black color change in Half Fraser broth indicating growth of *Listeria* sp. This occurred in samples previously exposed to cleaning and disinfection agents as well as heat and UV-C. Laboratory studies have shown that *L. monocytogenes* subjected to several types of stress, including heat, alkaline and acid stress can exhibit prolonged lag phases before growth in enrichment media (Bannenberg et al., 2021; Dupont & Augustin, 2009). These findings have practical implications for food processing environments, where *L. monocytogenes* may encounter multiple stressors, including chemical and UV-C exposure and thermal processes. Since food industry samples are typically analyzed using standardized protocols such as ISO 11290-1 (ISO, 2017), there is a potential risk of false-negative results if stressed cells fail to recover within the prescribed incubation time. This highlights the need to consider stress-induced growth delays when interpreting results and suggests that protocol adjustments or complementary detection methods may be warranted to improve reliability.

5. Conclusions

In conclusion, recommended user concentrations of C&D and UV-C treatment had limited effect against *L. monocytogenes* on the woven conveyor belts. Although extreme concentration of cleaning agents can significantly increase the effect, such concentrations may not be sufficient to completely eliminate *L. monocytogenes* and are not suitable for daily use. Heat treatment of the contaminated conveyor belts in a water bath at 60 °C for 1 h eliminated *L. monocytogenes*. Heat treatment with hot water has potential for increased use in the food industry for eliminating persisting *L. monocytogenes* from sites difficult to reach by chemical C&D and UV-C, provided that material compatibility and practical implementation constraints are carefully considered.

CRediT authorship contribution statement

Trond Møretrø: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Even Heir:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Tove Maugesten:** Writing – review & editing, Methodology, Investigation. **Diane Rip:** Writing – review & editing, Writing – original draft, Conceptualization. **Kyle Corbett:** Writing – review & editing, Writing – original draft, Conceptualization. **Edward M. Fox:** Writing – review & editing, Writing – original draft, Conceptualization. **Paula Teixeira:** Writing – review & editing, Writing – original draft, Conceptualization. **Solveig Langsrud:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2026.112320.

Data availability

Data will be made available on request.

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